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Antioxidant Activity and Phenolic Compounds in Selected Herbs

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The antioxidant capacities (oxygen radical absorbance capacity, ORAC) and total phenolic contents in extracts of 27 culinary herbs and 12 medicinal herbs were determined. The ORAC values and total phenolic contents for the medicinal herbs ranged from 1.88 to 22.30 μmol of Trolox equivalents (TE)/g of fresh weight and 0.23 to 2.85 mg of gallic acid equivalents (GAE)/g of fresh weight, respectively. *Origanum* × *majoricum*, *O. vulgare* ssp. *hirtum*, and *Poliomintha longiflora* have higher ORAC and phenolic contents as compared to other culinary herbs. The ORAC values and total phenolic content for the culinary herbs ranged from 2.35 to 92.18 μmol of TE/g of fresh weight and 0.26 to 17.51 mg of GAE/g of fresh weight, respectively. These also were much higher than values found in the medicinal herbs. The medicinal herbs with the highest ORAC values were *Catharanthus roseus*, *Thymus vulgaris*, *Hypericum perforatum*, and *Artemisia annua*. A linear relationship existed between ORAC values and total phenolic contents of the medicinal herbs ($R = 0.919$) and culinary herbs ($R = 0.986$). High-performance liquid chromatography (HPLC) coupled with diode-array detection was used to identify and quantify the phenolic compounds in selected herbs. Among the identified phenolic compounds, rosmarinic acid was the predominant phenolic compound in *Salvia officinalis*, *Thymus vulgaris*, *Origanum* × *majoricum*, and *P. longiflora*, whereas quercetin-3-*O*-rhamnosyl-(1 → 2)-rhamnosyl-(1 → 6)-glucoside and kaempferol-3-*O*-rhamnosyl-(1 → 2)-rhamnosyl-(1 → 6)-glucoside were predominant phenolic compounds in *Ginkgo biloba* leaves.

Keywords: *Antioxidant; phenolics; medicinal herbs; culinary herbs*

INTRODUCTION

Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidizing chain reactions (*1*). The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (*2*). In general, there are two basic categories of antioxidants, natural and synthetic. Recently, interest has increased considerably in finding naturally occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants, which are being restricted due to their carcinogenicity (*3*). Herbs have been used for a large range of purposes including medicine, nutrition, flavorings, beverages, dyeing, repellents, fragrances, cosmetics, charms, smoking, and industrial uses. Since prehistoric times, herbs were the basis for nearly all medicinal therapy until synthetic drugs were developed in the nineteenth century. Today, herbs are still found in 40% of prescription drugs (*4*). Culinary herbs have been grown and used for hundreds of years, and they are becoming increasingly popular in the United States for their ability to enhance and complement the flavors of a wide variety of foods (*5*).

Even though a variety of herbs are known to be sources of phenolic compounds, their compositional data are insufficient (*6*). Moreover, various herbs along with vegetables and fruits contain numerous phytochemicals in addition to phenolic compounds, such as nitrogen compounds, carotenoids, and ascorbic acid (*1*, *7*). Many of these phytochemicals possess significant antioxidant capacities that are associated with lower incidence and lower mortality rates of cancer in several human cohort (*1*).

The purpose of this study was to (i) evaluate a variety of culinary and medicinal herbs that were growing in the same location and same conditions with respect to their total phenolic content and antioxidant activity to find new potential sources of natural antioxidants; (ii) evaluate the relationship between phenolic content and antioxidant activity; and (iii) develop chromatographic procedures to identify and quantify phenolic antioxidants in selected herbs by high-performance liquid chromatography (HPLC).

MATERIALS AND METHODS

Chemicals. 2′,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH) was purchased from Wako Chemicals USA Inc, (Richmond VA). (*R*)-Phycoerythrin (R-PE) was obtained from Sigma (St. Louis, MO). 6-Hydroxy-2,3,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was purchased from Aldrich (Milwaukee, WI). Acetonitrile, methanol, acetone, and water were of HPLC grade and were purchased from Baxter (Muskegon, MI). Authentic standards were obtained from Sigma Chemical Co. (St. Louis, MO), Fisher Scientific Co. (Pittsburgh, PA), and Indofine Chemical Co. (Somerville, NJ).

Sample Preparation. The 39 different herbs (Table 1), including medicinal and culinary herbs, were collected on September 2000 from the National Herb Garden, which is part

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Table 1. Total Phenolic Content and Antioxidant Activity (ORAC) in Various Herbal Extracts[a]

common name	botanical name	type	total phenolic[b] (mg of GAE/g of fresh weight)	ORAC[c] (μmol of TE/ g of fresh weight)
balsam pear	*Momordica charantia*	medicinal	0.43 ± 0.08	3.43 ± 0.11
creeping thyme	*Thymus praecox* ssp. *arcticus*	medicinal	1.81 ± 0.04	13.40 ± 0.12
feverfew	*Tanacetum parthenium*	medicinal	0.87 ± 0.06	10.07 ± 0.15
garden sage	*Salvia officinalis*	medicinal	1.34 ± 0.09	13.28 ± 0.40
garden thyme	*Thymus vulgaris*	medicinal	2.13 ± 0.11	19.49 ± 0.21
Madagascar periwinkle	*Catharanthus roseus*	medicinal	2.85 ± 0.11	22.30 ± 0.54
maidenhair tree	*Ginkgo biloba*	medicinal	1.57 ± 0.05	13.18 ± 0.24
peppermint	*Mentha* × *piperita*	medicinal	2.26 ± 0.16	15.84 ± 0.42
Saint John's wort	*Hypericum perforatum*	medicinal	2.78 ± 0.12	16.77 ± 0.22
sweet wormwood	*Artemisia annua*	medicinal	1.54 ± 0.06	15.69 ± 0.37
true aloe	*Aloe vera*	medicinal	0.23 ± 0.00	1.88 ± 0.05
valerian	*Valerian officinalis*	medicinal	1.78 ± 0.12	15.82 ± 0.61
caraway	*Carum carvi*	culinary	1.05 ± 0.00	10.65 ± 0.29
chives	*Allium schoenoprasum*	culinary	1.05 ± 0.05	9.15 ± 0.28
cuban oregano	*Plectranthus amboinicus*	culinary	0.34 ± 0.00	4.71 ± 0.14
dandelion	*Taraxacum officinale*	culinary	0.26 ± 0.02	2.35 ± 0.14
dill	*Anethum graveolens*	culinary	3.12 ± 0.06	29.12 ± 0.29
English lavender	*Lavandula angustifolia*	culinary	1.50 ± 0.13	16.20 ± 0.11
fennel	*Foeniculum vulgare*	culinary	0.68 ± 0.00	5.88 ± 0.09
Greek mountain oregano	*Origanum vulgare* ssp. *hirtum*	culinary	11.80 ± 0.60	64.71 ± 1.05
hard sweet marjoram	*Origanum* × *majoricum*	culinary	11.65 ± 0.29	71.64 ± 1.25
lemon balm	*Melissa officinalis*	culinary	1.26 ± 0.04	9.54 ± 0.23
lemon thyme	*Thymus* × *citriodorus*	culinary	1.78 ± 0.03	13.28 ± 0.33
lemon verbena	*Aloysia triphylla*	culinary	1.55 ± 0.10	17.38 ± 0.35
lovage	*Levisticum officinale*	culinary	2.63 ± 0.05	21.54 ± 0.35
Mexican oregano	*Poliomintha longiflora*	culinary	17.51 ± 0.22	92.18 ± 0.72
orange mint	*Mentha aquatica*	culinary	2.26 ± 0.10	19.80 ± 0.43
parsley	*Petroselinum crispum*	culinary	1.12 ± 0.01	11.03 ± 0.13
pineapple sage	*Salvia elegans*	culinary	1.31 ± 0.08	11.55 ± 0.42
purple amaranth	*Amaranthus cruentus*	culinary	3.41 ± 0.11	28.92 ± 0.21
rose geranium	*Pelargonium graveolens*	culinary	7.34 ± 0.36	38.75 ± 0.61
rosemary	*Rosmarinus officinalis*	culinary	2.19 ± 0.15	19.15 ± 0.63
salad burnet	*Sanguisorba minor*	culinary	0.99 ± 0.07	8.33 ± 0.13
society garlic	*Tulbaghia violacea*	culinary	1.03 ± 0.10	7.50 ± 0.60
spearmint	*Mentha spicata*	culinary	0.94 ± 0.15	8.10 ± 0.26
sweet basil	*Ocimum basilicum*	culinary	2.23 ± 0.15	14.27 ± 0.45
sweet bay	*Laurus nobilis*	culinary	4.02 ± 0.90	31.70 ± 0.97
Vietnamese coriander	*Polygonum odoratum*	culinary	3.09 ± 0.12	22.30 ± 0.68
winter savory	*Satureja montana*	culinary	3.16 ± 0.02	26.34 ± 0.17
$LSD_{0.05}$			0.27	0.79

[a] Data expressed as mean ± SEM. [b] Data expressed as milligrams of gallic acid (GAE) equivalents per gram of fresh weight. [c] Data expressed as micromoles of Trolox equivalents per gram of fresh weight.

of the U. S. National Arboretum in Washington, D.C. All the herbs were grown in the same location and same conditions to avoid variations of oxygen radical absorbance capacity (ORAC) values due to environmental factors. All samples were stored in a freezer at −80 °C before analysis. Herbs (2.0 g) were extracted with 15 mL of phosphate buffer (75 mM, pH 7.0) using a Polytron homogenizer (Brinkmann Instruments, Inc., Westbury, NY) for 1 min and were then centrifuged at 20000*g* for 20 min. The supernatant was recovered and used for the ORAC and total phenolic compound assay after suitable dilution with phosphate buffer (75 mM, pH 7.0).

Oxygen Radical Absorbance Capacity (ORAC) Assay. The procedure for performing ORAC assays for the herb samples were based on a previous report of Wang and Lin (*8*), which was modified from a method described by Cao et al. (*9*). This assay measures the ability of antioxidant compounds in test materials to inhibit the decline of R-PE fluorescence that is induced by a peroxyl radical generator, AAPH. The reaction mixture contained 1.7 mL of 75 mM phosphate buffer (pH 7.0), 100 μL of R-PE (3.4 mg/L), 100 μL of 320 nM AAPH, and 100 μL of sample. Trolox, a water-soluble analogue of vitamin E, was used as a control antioxidant standard. The fluorescence of R-PE was determined and recorded every 5 min at the excitation wavelength of 540 nm and emission wavelength of 570 nm using a Shimadzu RF-Mini 150 recording fluorometer (Columbia, MD) until the fluorescence of the last reading declined to <5% of the first reading. The final results (ORAC value) were calculated using the differences of areas under the quenching curves of R-PE between a blank and a sample and expressed as micromoles of Trolox equivalents (TE) per gram of fresh weight.

Total Phenolic Compound Analysis. The amount of total phenolics in the herb extracts was determined with the Folin-Ciocalteu reagent according to the method of Slinkard and Singleton (*10*) using gallic acid as a standard. Samples (200 μL, two replicates) were introduced into test cuvettes, and then 1.0 mL of Folin-Ciocalteu's reagent and 0.8 mL of Na_2CO_3 (7.5%) were added. The absorbance of all samples was measured at 765 nm using the Shimadzu UV−Vis spectrophotometer after incubating at 30 °C for 1.5 h. Results were expressed as milligrams of gallic acid equivalent (GAE) per gram of fresh weight.

HPLC Analysis of Selected Herbs. The sample (2.0 g) was extracted twice with 15 mL of acetone using a Polytron homogenizer (Brinkmann Instruments, Inc., Westbury, NY) for 1 min. The extract was centrifuged, and the residue was washed and agitated twice with 5 mL of solvent. The combined extract was evaporated to dryness under reduced pressure. The residue was dissolved in 4 mL of methanol, and 20 μL aliquots were analyzed by HPLC. The herb extracts used for HPLC analysis were passed through a 0.45-μm filter (Millipore, MSI, Westboro, MA) before injection into a reverse phase NOVA-PAK C_{18} column (150 × 39 mm i.d., particle size 4 μm) with a guard column (NOVA-PAK C_{18}, 20 × 3.9 mm i.d., particle size 4 μm) at ambient temperature (20 °C). A Waters 600E system controller coupled with a photodiode array detector (Waters 990 series) was used. The mobile phase was acetonitrile (A) and acidified water containing 2.5% formic acid

(B). The gradient was as follows: 0 min, 5% A; 10 min, 15% A; 30 min, 25% A; 35 min, 30% A; 50 min, 55% A; 55 min, 90% A; 57 min, 100% A and then held for 10 min before returning to the initial conditions. The flow rate was 1.0 mL/min and the wavelengths of detection were set at 280, 330, and 350 nm. Scanning between 200 and 450 nm was performed, and the data were collected by the Water 990 3-D chromatography data system. This mobile phase (gradient 1) was used to analyze most of the herbs, including *Salvia officinalis, Rosmarinus officinalis, Thymus vulgaris, Origanum* × *mejoricum*, and *Poliomintha longiflora*.

For determination of the phenolic compounds in the herb *Ginkgo biloba*, gradient 2 was used with acetonitrile (A) and acidified water containing 2.5% formic acid (B) as the solvent system. The gradient was as follows: 0 min, 5% A; 10 min, 15% A; 30 min, 25% A; 40 min, 100% A, and then held 10 min before returning to the initial conditions. Identification of the individual flavonoids was based on the comparison of the retention times and the UV spectrum obtained by photodiode array (PDA) of unknown peaks to those of reference authentic standards.

Data Analysis. Correlation and regression analyses of ORAC activity (Y) versus the total phenolic content (X) were carried out using the regression program in Microcal Origin (Microcal Software Inc., Northampton, MA). Data were subjected to analysis of variance, and means were compared by least significant difference (LSD) used in NCSS (*11*). Differences at $p < 0.05$ were considered to be significant.

RESULTS AND DISCUSSION

Antioxidant Activity and Total Phenolic Content. The antioxidant capacity (expressed as ORAC value) and total phenolic content of all extracts, including culinary and medicinal herbs, are shown in Table 1. Since most culinary herbs are prepared for consumption using aqueous methods, herbal antioxidant levels were prepared for measurement by aqueous, rather than organic, extraction to approximate the levels of antioxidants in herbs that might be consumed and absorbed during normal dietary intake.

The ORAC values for the medicinal herbs ranged from 1.88 μmol of Trolox equivalents to 22.3 μmol of TE/g of fresh weight. The phenolic concentration of the medicinal herbs ranged from 0.23 mg of gallic acid equivalents (GAE)/g of fresh weight to 2.85 mg of GAE/g of fresh weight. The medicinal herbs with the highest ORAC values were *Catharanthus roseus* (22.30 μmol of TE/g of fresh weight), *T. vulgaris* (19.49 μmol of TE/g of fresh weight), *Hypericum perforatum* (16.77 μmol of TE/g of fresh weight), and *Mentha* × *piperita* (15.84 μmol of TE/g of fresh weight). *C. roseus* (Madagascar periwinkle) contains the alkaloids, vinblastine and vincristine, which are extracted from the dried whole plant and widely used in the treatment of leukemia, lymphomas, and cancer (*4*). The fresh or dried leaves and flowers of *H. perforatum* (Saint John's Wort) have traditionally been used externally to treat wounds and burns, and internally as a tonic, antidepressant, and tranquilizer (*4*). *T. vulgaris* (garden thyme) is employed topically in lotions, creams, and ointments because of its antibacterial and antifungal action (*4*). *Mentha* × *piperita* (peppermint) is a natural hybrid, and a tea of the leaves is used primarily for its gas-relieving properties in treating colic, indigestion, and flatulence (*4*). These four herbs also had the highest phenolic content: 2.85 mg of GAE/g of fresh weight, 2.13 mg of GAE/g of fresh weight, 2.78 mg of GAE/g of fresh weight and 2.26 mg of GAE/g of fresh weight, respectively. *Aloe vera* had the lowest ORAC value (1.88 μmol of TE/g of fresh weight) and phenolic concentration (0.23 mg of GAE/g of fresh weight). The gel obtained from freshly cut leaves of *A. vera* can be applied to the skin for minor burns and irritations (*4*). The relationship between the ORAC (Y) and phenolic contents (X) of all medicinal herbs had a correlation coefficient of 0.919 ($Y = 6.10X + 2.64$). There was a positive linear correlation between the phenolic content and antioxidant capacity of the medicinal herbs.

The ORAC values for the culinary herbs ranged from 2.35 to 92.18 μmol of TE/g of fresh weight. The phenolic concentration of the culinary herbs ranged from 0.26 to 17.51 mg of GAE/g of fresh weight. This was much higher than that of the medicinal herbs. The culinary herbs *P. longiflora*, *Origanum* × *majoricum*, and *O. vulgare* ssp. *hirtum* had high ORAC values and phenolic contents (Table 1). These three herbs are also known as Mexican oregano, Italian oregano, and Greek mountain oregano, respectively. The Italian oregano is a versatile herb used to season meats, egg dishes, soup, and vegetables (*5*). The Greek mountain oregano is known for its pepper-flavored leaves that provide a piquant flavor associated with Italian pizza and classic Greek cuisine (*5*). The relationship between the ORAC (Y) and phenolic contents (X) of the culinary herbs had a correlation coefficient of 0.986 ($Y = 5.19X + 5.66$). The overall correlation coefficient in herbs (culinary and medicinal herbs) between ORAC (Y) and phenolic contents (X) was 0.984 ($Y = 5.23X + 5.32$). These results indicated that the phenolic compounds had a major contribution to the antioxidant capacity of herbs.

Analysis of Phenolic Compounds in Selected Herbs. Typical phenolics that possess antioxidant activity are known to be mainly phenolic acids and flavonoids (*6*). Phenolic acids have been repeatedly implicated as natural antioxidants in fruits, vegetables, and other plants. For example, caffeic acid, ferulic acid, and vanillic acid are widely distributed in the plant kingdom. Caffeic acid has been found to have high activity comparable to that of the flavonoid, quercetin (*7*). Ferulic acid was shown to inhibit the photoperoxidation of linoleic acid at the somewhat high concentration of 10^{-3} M (*7*). The most widespread and diverse phenolics are the flavonoids which have the same C_{15} ($C_6-C_3-C_6$) skeleton and possess antioxidant capacity toward a variety of easily oxidizable compounds (*12*). In many herbs, the main flavonoid constituents are flavonol aglycones such as quercetin, myricetin, kaempferol, and their glycosides (*6*). In general, flavonoids containing multiple hydroxyl groups have higher antioxidant activities against peroxyl radicals than do phenolic acids. However, the flavonoid glycosides (including rutin, naringin, and hesperidin) usually have low ORAC values (*12*).

Selected phenolics in several herbs, separated and identified by using reversed-phase high-performance liquid chromatography (HPLC), are presented in Table 2 and Figure 1 (chemical structures). Considerable variation was found in phenolic compounds of different herbs.

Sage (*S. officinalis*) is not only widely used as a natural source of food flavoring but also has medicinal properties for the treatment of various diseases (*16*). The HPLC analysis of sage extracts showed that a large number flavonoids and phenolic acids were present in significant amounts (Figure 2 and Table 2). Rosmarinic acid (117.8 mg/100 g of fresh weight) and luteolin (33.4 mg/100 g of fresh weight) were the most abundant

Table 2. Phenolic Compounds in Some Selected Herbs (mg/100 g of fresh weight)[a]

phenolic compound	*Salvia officinalis*	*Ginkgo biloba*	*Origanum* × *majoricum*	*Poliomintha longiflora*	*Thymus vulgaris*	*Rosmarinus officinalis*
vanillic acid	2.27 ± 0.48	1.45 ± 0.04		3.59 ± 0.08		1.73 ± 0.08
caffeic acid	7.42 ± 0.35	39.8 ± 2.31	10.4 ± 0.27	8.1 ± 0.09	11.7 ± 1.04	2.95 ± 0.12
luteolin	33.4 ± 1.32			25.1 ± 0.76	39.5 ± 1.53	
rosmarinic acid	117.8 ± 1.01		154.6 ± 3.29	124.8 ± 3.57	91.8 ± 2.75	32.8 ± 1.69
hispidulin	16.3 ± 1.07		48.7 ± 1.58	10.8 ± 1.15	20.8 ± 0.96	19.7 ± 1.12
cirsimaritin	14.3 ± 0.83					24.4 ± 0.87
carnosic acid						126.6 ± 6.00
apigenin	2.4 ± 0.07		3.5 ± 0.11			1.1 ± 0.15
naringin (naringenin-5-rhamnosidoglucoside)						53.1 ± 2.09
rosmanol						124.1 ± 3.19
rutoside (quercetin-3-rutinoside)		22.4 ± 1.57				
quercetin-3-*O*-rhamnosyl-(1 → 2)-rhamnosyl-(1 → 6)-glucoside[b]		77.9 ± 3.25				
kaempferol-3-*O*-rhamnosyl-(1 → 2)-rhamnosyl-(1 → 6)-glucoside[b]		75.6 ± 2.18				

[a] Data expressed as mean ± SEM. [b] Data expressed as milligrams equivalents of rutoside per 100 g of fresh weight.

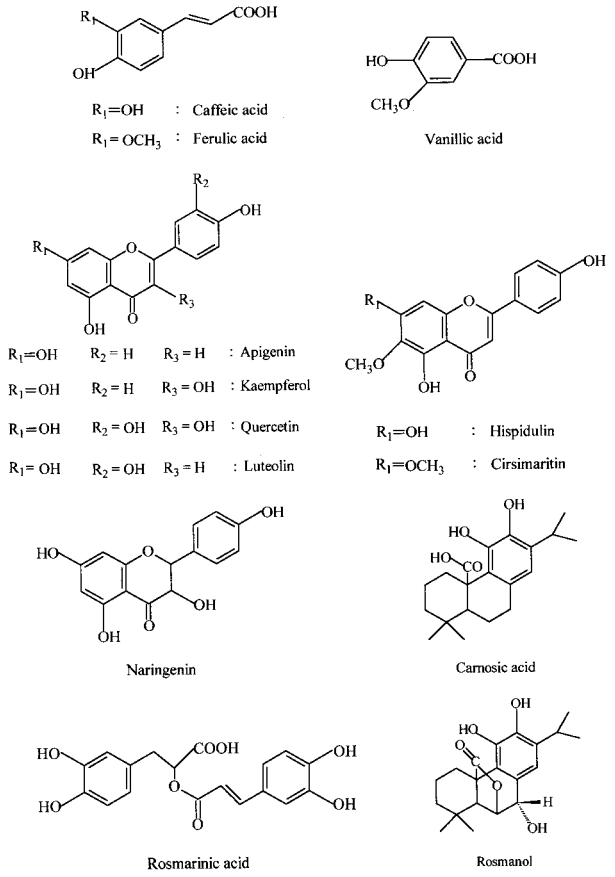

Figure 1. Structures of phenolic compounds in some selected herbs.

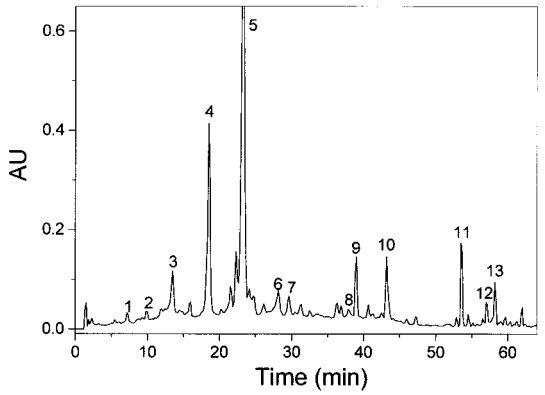

Figure 2. HPLC profile of *S. officinalis* herbal extract. (1) Vanillic acid; (2) caffeic acid; (3) ferulic acid; (4) luteolin 7-*O*-glucoside; (5) rosmarinic acid; (6) 4′,5,7,8-tetrahydroxyflavone; (7) scutellarein; (8) apigenin; (9) hispidulin; (10) cirsimaritin; (11) carnosol; (12) carnosic acid; (13) methyl carnosate. Detection at 280 nm.

phenolic constituents in the extracts and were readily identified by comparison with authentic standards. Other compounds with characteristic spectra of vanillic acid, caffeic acid, ferulic acid, luteolin 7-*O*-glucoside, rosmarinic acid, 4′,5,7,8-tetrahydroxyflavone, scutellarein, apigenin, hispidulin, cirsimarin, carnosol, carnosic acid, and methyl carnosate were detected, and the occurrence of these phenolics was confirmed by published reports of chromatographic and UV spectra data (*13*−*16*). In addition, many volatile constituents of sage have been studied such as 1,8-cineole, thujone, isothujone, and camphor (*17*).

In early pharmacological works, the extracts of sage showed multiple biological activities including antimutagen, antiviral, and antioxidant activity (*14*). Cuvelier et al. (*14*) measured the correlation between antioxidant efficiency and the composition of sage and recognized that carnosol, rosmarinic acid, and carnosic acid had the greatest antioxidant activities followed by caffeic acid and cirsimaritin. Vanillic acid had only half of the antioxidant activity of caffeic acid, and the relative antioxidant activities among caffeic acid, luteolin, and apigenin was 1.3, 2.1, and 1.5 (*12*).

Ginkgo biloba leaf extract, which is a complex product containing different active compounds (mainly flavonoids and terpenes), is used as a phytomedicine to increase peripheral and cerebral blood flow (*20*). The HPLC analysis of *G. biloba* leaf extract is shown in Figure 3. Some phenolic compounds were identified by comparison with authentic standards together with reports of Pietta et al. (*18*, *19*), Hasler et al. (*20*), and Ellnain-Wojtaszek and Zgórka (*21*). From Figure 3 and Table 2, caffeic acid (39.8 mg/100 g of fresh weight), quercetin-3-*O*-rhamnosyl-(1 → 2)-rhamnosyl-(1 → 6)-glucoside (77.9 mg/100 g of fresh weight) and kaempferol-3-*O*-rhamnosyl-(1 → 2)-rhamnosyl-(1 → 6)-glucoside (75.6 mg/100 g of fresh weight) were major components in the extract. These three components have exhibited high antioxidant activities with ORAC values of 2.23, 3.29, and 2.67, respectively (*12*). A great variety of flavonoid glycosides was also found in *G. biloba* leaf extract (*20*). Its characteristic constituents were bifla-

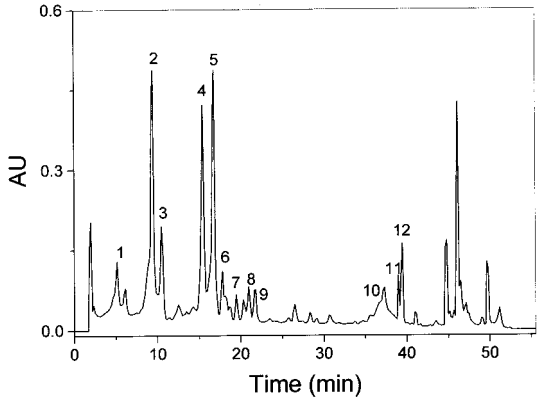

Figure 3. HPLC profile of *Ginkgo biloba* herbal extract. (1) vanillic acid; (2) caffeic acid; (3) isovanillic acid; (4) quercetin-3-*O*-rhamnosyl-(1 → 2)-rhamnosyl-(1 → 6)-glucoside; (5) kaempferol-3-*O*-rhamnosyl-(1 → 2)-rhamnosyl-(1 → 6)-glucoside; (6) rutoside; (7) isoquercitrin; (8) kaempferol-3-*O*-rutinoside; (9) kaempferol-glycoside; (10) bilobetin; (11) ginkgetin; (12) isoginkgetin. Detection at 330 nm.

vones (bilobetin, ginkgetin, isoginkgetin), vanillic acid, and rutoside, which possess medicinal value and antioxidant activities (*20*).

Oregano belongs to the *Lamiaceae* family of herbs and has been extensively studied as an effective antioxidant in the lipid system (*22*). In this study, we found that certain species of Oregano (*P. longiflora*, *Origanum vulgare* ssp. *hirtum* and *Origanum* × *majoricum*) had extremely high total phenolic contents and ORAC values (Table 1). Their antioxidant activities were higher than α-tocopherol and were comparable to that of BHA against linoleic acid oxidation (*23*). The HPLC analysis of *Origanum* × *majoricum* and *P. longiflora* leaf extracts are shown in Figure 4 (panels A and B). *Oregano* species extracts had high contents of rosmarinic acid (124.8∼154.6 mg/100 g of fresh weight) and other hydroxycinnamic acid compounds (Table 2). Rosmarinic acid and hydroxycinnamic acid compounds have been demonstrated to possess strong antioxidant activity (*7*, *24*). The antioxidant activity of rosmarinic acid is much higher than that of α-tocopherol and BHT (*24*).

The herbs thyme (*T. vulgaris*) and rosemary (*R. officinalis*) are known to have high antioxidant capacities (*25*), and some methylated flavones were isolated from the thyme herb as antioxidants (*26*). In the essential oil of thyme, thymol and carvacrol were recognized as major components that showed high antioxidant and antimicrobial activity (*6*, *27*). In addition, a biphenyl compound (3,4,3′,4′-tetrahydroxy-5,5′-diisopropyl-2,2′-dimethylbiphenyl) and a flavonoid (eriodicytol) have also been isolated from thyme and reported to be potent antioxidants inhibiting superoxide anion production in the xanthine/xanthine oxidase system and mitochondrial and microsomal lipid peroxidation (*28*). The biphenyls, dimers of thymol, and flavonoids isolated from thyme showed antioxidant activity as strong as BHT (*23*). In the present study, high contents of rosmarinic acid (91.8 mg/100 g of fresh weight) and luteolin (39.5 mg/100 g of fresh weight) were found in the extract of thyme. The extracts of rosemary were the first marketed natural antioxidants. Several phenolic compounds of rosemary determined in this study were similar in content and concentration to those in previous reports (*14*), i.e., rosmanol (124.1 mg/100 g of fresh weight), rosmarinic acid (32.8 mg/100 g of fresh weight), naringin (53.1 mg/100 g of fresh

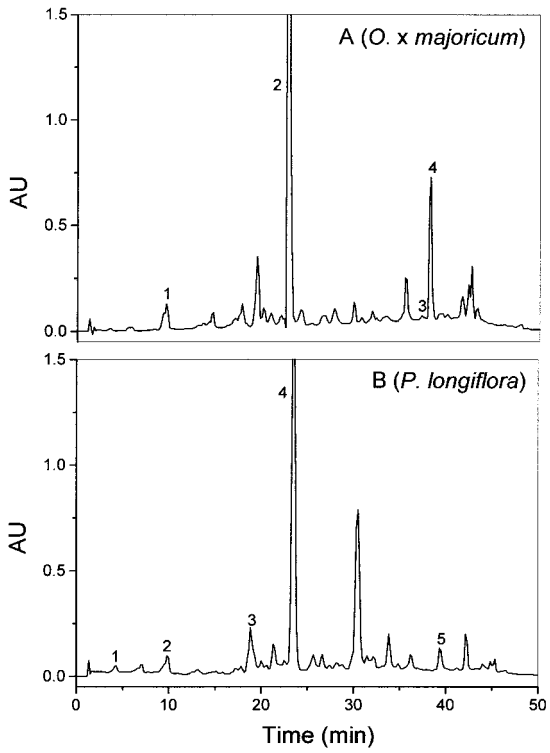

Figure 4. HPLC profile of herbal extracts. (A) *Origanum* × *majoricum*: (1) caffeic acid; (2) rosmarinic acid; (3) apigenin; (4) hispidulin. (B) *Poliomintha longiflora*: (1) vanillic acid; (2) caffeic acid; (3) luteolin; (4) rosmarinic acid; (5) hispidulin. Detection at 330 nm.

weight), cirsimaritin (24.4 mg/100 g of fresh weight), and carnosic acid (126.6 mg/100 g of fresh weight). Similar to sage, these phenolic compounds in rosemary extracts are very potent antioxidants and are utilized in many food products. Rosmanol is an active antioxidant and has more activity than α-tocopherol or BHT (*23*). As compared with the commercial antioxidants BHA and BHT, the phenolic antioxidants from rosemary provide desirable flavors in frying operations (*29*). Rosmarinic acid has been shown to possess more antioxidant activity than rosmanol (*14*).

The present study showed that due to the diversity and complexity of the natural mixtures of phenolic compounds in the various herb extracts, it is rather difficult to characterize every compound and assess or compare their antioxidant activities. Each herb generally contained different phenolic compounds, and each of these compounds possessed differing amounts of antioxidant activity. The antioxidant activities of flavonoids increased with the number of hydroxyl groups substituted on to the B-ring, especially at C-3′, and a single hydroxy substituent generates little or no additional antioxidant capacity (*30*). There were also some antioxidant activities in herbs that may be attributable to other unidentified substances or to synergistic interactions. The total phenolic contents and ORAC values in many herbs in this study were higher than reported for berries, fruits, and vegetables (*8*, *31*). There was a positive linear correlation between the phenolic content and antioxidant capacity of the herbs. This study revealed that herbs are an effective potential source of natural antioxidants. Therefore, supplementing a balanced diet with herbs may have beneficial health effects.

ABBREVIATIONS USED

AAPH, 2′,2′-azobis(2-amidinopropane) dihydrochloride; ORAC, oxygen radical absorbance capacity; R-PE, (*R*)-phycoerythrin; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; TE, Trolox equivalents.

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